Pharmacology Biochemistry & Behavior, Vol. 12, pp. 297–301. Printed in the U.S.A.

Effect of Acute Ethanol Ingestion on Integrated Plasma Prolactin Levels in Normal Men

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Received 4 September 1979

ELLINGBOE, J., J. H. MENDELSON, J. C. KUEHNLE, A. S. T. SKUPNY AND K. D. MILLER. *Effect of acute ethanol ingestion on integrated plasma prolactin levels in normal men.* PHARMAC. BIOCHEM. BEHAV. 12:(2) 297–301, 1980.—Healthy male subjects ingested 1.0 g ethanol/kg (Alcohol Day) and caloric equivalents of sucrose (Control Day). Plasma prolactin was determined on samples collected at 20-min intervals by serial constant blood exfusion, from 2 hr before to 4 hr after the drink. In 14 of the 15 men studied, plasma prolactin levels during the 2-hr period after alcohol administration were elevated an average of 31% above values for the preceding 2-hr period. Data pooled for all subjects revealed a small but statistically significant increase in prolactin coinciding with ascending and peak concentrations of blood alcohol. A significant increment in prolactin was associated with peak blood alcohol levels when values were compared between control and alcohol treatment days. Although of statistical significance, these transient and variable increases were within the normal range of basal prolactin levels for most subjects and are unlikely to be physiologically meaningful.

Alcohol Ethanol Prolactin Man

ELEVATED plasma prolactin levels have recently been described in certain chronic alcoholics [11,12]. Related to these findings is the earlier suggestion that alcohol might stimulate prolactin secretion [13]. Other investigators, however, have been unable to find evidence to support the hypothesis that either alcohol per se or chronic alcoholism might enhance prolactin secretion [2, 4, 10, 14, 15].

Prompted by a report of alcohol stimulation of prolactin release from perfused isolated rat pituitary cells [7], we undertook a reevaluation of the prolactin response to acute alcohol administration in healthy non-alcoholic men, using each subject as his own control and collecting blood by a serial integrated sampling technique.

METHOD

Data were obtained on 15 normal male volunteers, 22 to 30 years of age, with an average age of 26 years. All were in good health, with no evidence of sexual dysfunction or liver disease. They had no past or current history of alcohol or drug abuse and had used no medication within one week prior to the study. Each subject was given a complete physical and mental status examination and the following laboratory tests were conducted: LDH, SGOT, total bilirubin, total protein, albumin, gamma globulin, protein electrophoresis, alkaline phosphatase, calcium, phosphorous, sodium, potassium, chloride, uric acid, urea nitrogen (BUN) creatinine, glucose, total cholesterol, WBC, RBC, hemoglobin, hematocrit and WBC differential. An abnormal finding in any of these assessments was sufficient to disqualify a potential subject. The study was approved by the Human Studies Committee and Institutional Review Board of the McLean Hospital. Signed informed consent was obtained from each subject before experimental procedures were initiated.

The subjects were asked to fast overnight before each of two study days—a control and an alcohol ingestion day, scheduled one week apart. Alcohol was administered as 100 proof beverage (vodka) in a dose of 2.5 ml/kg body weight (1.0 g/kg) diluted to a total volume of 360 ml with fruit juice. This amount of ethanol was selected to produce a peak blood alcohol level of at least 100 mg/dl, which is associated with mild to moderate intoxication, and is equivalent to approximately 4 drinks (1.5 oz) of distilled spirits. Subjects 1 through 7 received ethanol on the first study day, while Subjects 8 through 15 were given sweetened fruit juice on the first study day and alcohol on the second study day. Subjects consumed their drinks within 15 min.

On each of the two study days the subjects were catheterized in an antecubital vein and 20-min serial integrated samples of blood were collected over a 6-hr period (2 hr before and 4 hr after ingestion of the drink) by means of portable non-thrombogenic constant exfusion pumps (Sigmamotor ML-6-3 Constant Blood Withdrawal Pump, Cormed, Inc., Middleport, NY). Hormone concentrations measured in continuously collected blood samples can be considered to be true mean values (integrated) for the sampling period [1]. During blood collection the subjects were fully ambulatory and relatively unrestricted in physical activity. Heparinized plasma samples were prepared and frozen immediately, then stored below −20° C for subsequent analysis. Immediately following removal of each blood

sample, blood ethanol levels were determined using a calibrated apparatus (Breathalyzer, Model 900A, Stephenson Corporation, Eatontown, NJ).

Plasma prolactin concentrations were measured in duplicate 0.100-ml aliquots using a double antibody radioimmunoassay similar to that described by Midgley for human gonadotropins [5]. Radioiodinated prolactin was purchased from New England Nuclear (Boston, MA). Standard prolactin (VLS 3, donated by Drs. Vanderlaan, Lewis and Sinha) and antiserum (rabbit AFP anti-hPRL-1, prepared by Dr. Parlow) were provided by the National Pituitary Agency (University of Maryland School of Medicine), National Institute of Arthritis, Metabolism and Digestive Diseases. Prolactin results are expressed as nanograms VLS 3 prolactin standard per milliliter plasma. As used in our laboratory the assay sensitivity was 3 ng/ml. The intra-assay coefficients of variation were 6.9% and 3.1% for samples in the 5–10 ng/ml and 25–30 ng/ml ranges, respectively. Inter-assay coefficients of variation were 9.1% and 3.2% for control samples averaging 3.9 ng/ml and 26.8 ng/ml, respectively. All samples from an individual subject were analyzed in the same assay. Data were evaluated statistically by using the *t*-test (paired, 2-tailed), two-way repeated measures analysis of variance and Fisher LSD test for multiple comparisons.

To assess the possibility of ethanol interference with the prolactin radioimmunoassay, 10 replicate aliquots of high, medium and low control plasma samples were analyzed with and without added ethanol (0, 100 and 400 mg ethanol/dl plasma). The same concentrations of ethanol were added to the serially diluted prolactin standard curve analyzed in triplicate. Prolactin concentrations determined in the presence and absence of ethanol were identical.

RESULTS

Data obtained in this investigation revealed an increase in plasma prolactin in 14 of the 15 subjects studied when mean prolactin values for the 2-hr period following alcohol consumption were compared with mean values for the preceding 2-hr period. The increase in prolactin associated with ethanol ingestion during the entire 2-hr period of ascending and peak blood ethanol averaged 31% (±8.3 SE) with a range of +3.6% to +85.1%.

Plasma prolactin levels on control and alcohol treatment days are depicted for each of the fifteen subjects in Fig. 1. The height of each bar represents the 20-min mean integrated prolactin concentration. Solid black bars depict a 1-hr period of peak blood alcohol levels, while diagonally striped bars indicate prolactin concentrations for the ascending and descending limbs of the blood alcohol curve.

Data obtained for most subjects on the control day were consistent with expected normal basal prolactin levels. A rise of prolactin in Subject 5 near the end of the catheterization period was associated with catheter problems, while a ten-fold elevation of plasma prolactin in Subject 14 occurred when the catheter became completely occluded, necessitating reinsertion of a fresh catheter.

During the predrinking period on the day of alcohol administration, prolactin levels were similar to those observed throughout the control day (Subjects 5 and 14 excluded).

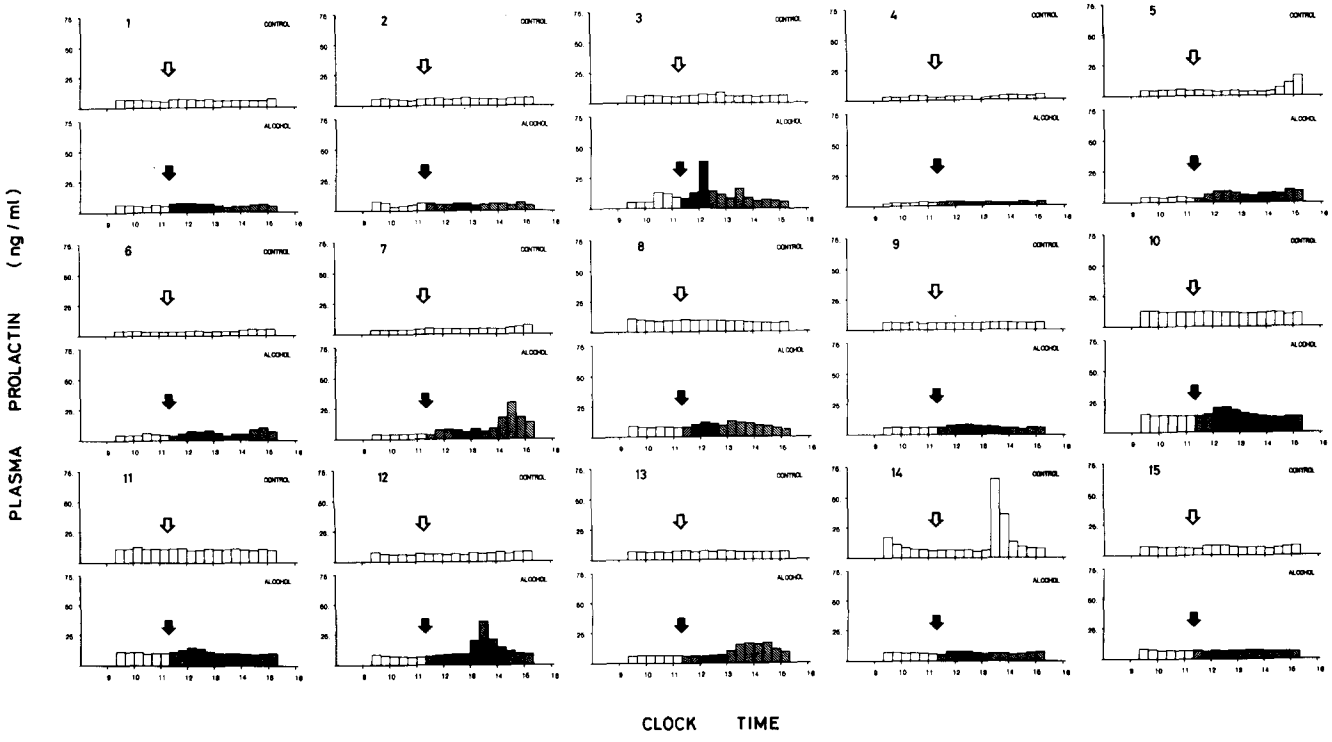

FIG. 1. Plasma prolactin levels of individual subjects, as measured in serial 20-min integrated samples over 6-hr intervals on control and alcohol administration days. The time of administration of alcohol (1.0 g/kg body weight) or isocaloric amounts of sucrose is shown by the arrow. A 1-hr period of peak blood ethanol is indicated by solid bars, while ascending and descending blood alcohol periods are designated by diagonally striped bars.

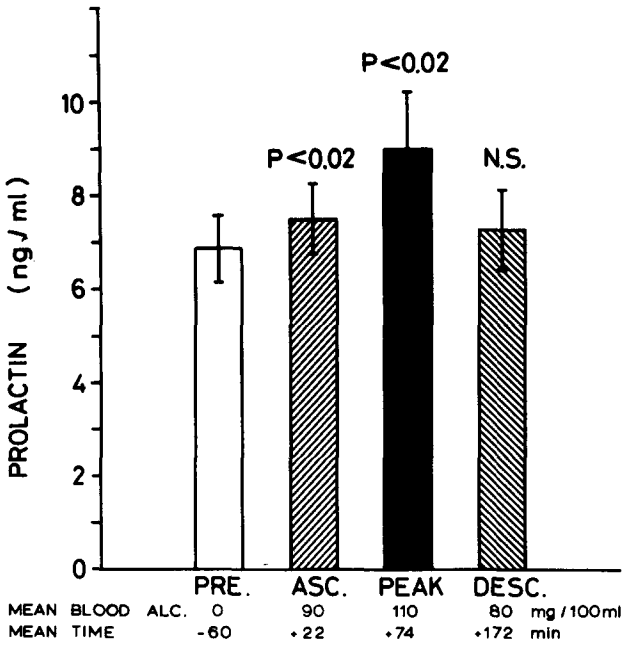

FIG. 2. Mean (± SE) plasma prolactin levels on alcohol administration day, for predrinking period and the ascending, peak and descending phases of the blood alcohol curve, as designated in Fig. 1. Statistical significance (t-test) between predrinking and postdrinking phases is indicated above the relevant bars.

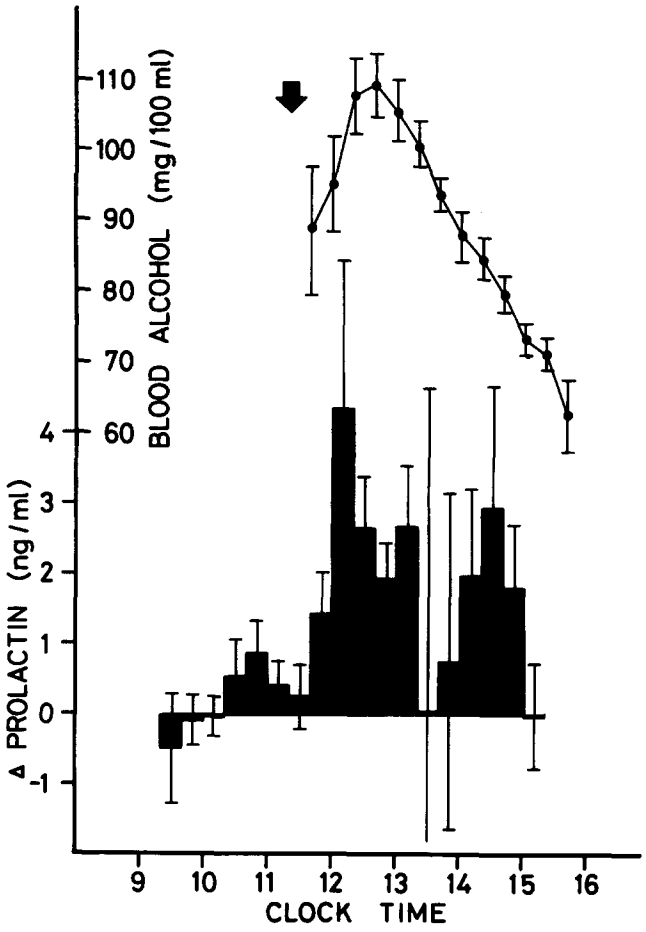

FIG. 3. Mean (± SE) blood alcohol concentrations and differences in plasma prolactin levels (alcohol day values minus control day values) for all 15 subjects. Two-way repeated measures analysis of variance indicated significantly higher prolactin levels ($p<0.01$) during the period from 20 min to 120 min following ethanol administration.

Following alcohol ingestion, however, a modest increment of prolactin was apparent during ascending and peak phases of the blood alcohol curve. After blood alcohol levels had peaked, during the descending phase of the curve, four subjects (7, 8, 12 and 13) became nauseated, vomited, and slept intermittently. At the onset of nausea in these four subjects there was a corresponding marked rise in plasma prolactin.

Data pooled and averaged over four time periods on the alcohol administration day are depicted in Fig. 2. When mean plasma prolactin levels during the 1-hr period of peak blood alcohol are compared with the corresponding predrinking prolactin values, the prolactin increment is 31%. By excluding Subject 3, who exhibited an unusually rapid rise in blood alcohol and the greatest increase in plasma prolactin, an average increment of 27% is calculated for the peak blood alcohol period. Evaluation of these data for all subjects using a paired t-test indicates that the prolactin increment relative to predrinking levels is statistically significant (peak vs predrinking: mean=+31%, $t(14)=2.76$, $p<0.02$). When Subject 3 is excluded the prolactin increment is still significant (ascending vs predrinking: mean=+10%, $t(13)=2.69$, $p<0.02$; peak vs predrinking: mean=+27%, $t(13)=3.46$, $p<0.005$). During the descending blood alcohol period Subjects 7, 8, 12 and 13 were excluded from calculations because of spuriously high prolactin values associated with nausea. For the remaining subjects there is no significant difference between the descending blood alcohol and predrinking periods.

In Fig. 3 differences in plasma prolactin levels between control and alcohol administration days are presented for all subjects (no data excluded) as means (± SE) for each 20-min interval vs clock time. This figure illustrates the time course of the rise in both blood ethanol and plasma prolactin, emphasizing the great variance among subjects especially during the descending limb of the blood alcohol curve. Statistical assessment by analysis of variance indicates that significant increments in the level of prolactin are associated only with the highest blood alcohol levels (95–110 mg/100 ml), from 20 min to 2 hr after ethanol ingestion. The intensity of intoxication and enhanced arousal, as estimated by the Profile of Mood States (POMS) self report test were also correlated positively with peak blood alcohol levels and increased plasma prolactin concentrations. These data will be reported separately.

DISCUSSION

Prior to William's suggestion [13] that alcohol might stimulate prolactin secretion and thereby increase the risk of breast cancer, Toro and his colleagues [10] had been unable to demonstrate any effects of acute alcohol administration on prolactin when measured in samples taken 0, 30, 60 and 180 min after ethanol ingestion. Ylikahri and coworkers [14,15]

responded to William's hypothesis by reporting that alcohol intake by healthy male volunteers did not enhance significantly the thyroliberin-induced prolactin release during the period of intoxication. Moreover, they observed almost complete abolition of the prolactin response to thyroliberin during hangover. Loosen and Prange [4] also measured both basal and thyroliberin-induced prolactin in normal subjects and in alcoholics, during and after withdrawal. They found no significant differences due to alcohol other than a reduction in basal prolactin levels during withdrawal in alcoholic men. Earll *et al.* [2] also measured basal prolactin levels in blood samples collected at half-hour intervals, from 30 min before to 180 min after ethanol ingestion by five normal males; no significant change in plasma prolactin was found when comparing preadministration to post-alcohol administration periods. The weight of published data thus indicates that neither acute alcohol administration to normal subjects, nor alcoholism per se cause significant perturbation of plasma prolactin levels, but that either basal or thyroliberin-stimulated prolactin may be lower than normal during hangover or alcoholic withdrawal.

Nevertheless, there is now good evidence of increased plasma prolactin levels in a subgroup of alcoholic men who exhibit both irreversible alcoholic cirrhosis and gynecomastia [11,12]. Such men were reported to have mean basal prolactin levels averaging about 4.5 times the normal basal prolactin levels of healthy non-alcoholic subjects, alcoholics with fatty liver and cirrhotics without gynecomastia. Not only were basal prolactin levels elevated in this group, but plasma prolactin reached higher peak concentrations following thyroliberin administration, although the percentage increase in prolactin response above baseline was significantly lower than normal. Conversely, basal prolactin levels were somewhat below normal in alcoholics with fatty liver (reversible liver disease), but the percentage increase in prolactin response to thyroliberin in these subjects averaged about 2.5 times the response of normal healthy volunteers. Van Thiel and his colleagues suggest that these findings indicate progressive stages of alcohol-induced hypothalamic-pituitary dysfunction [12].

Our results indicate that acute alcohol intake in healthy male subjects does indeed result in statistically significant elevations of plasma prolactin during the period of peak blood ethanol levels. Nevertheless, the increment in plasma prolactin following a single intoxicating dose of alcohol in most subjects was transient and within the normal range of basal prolactin levels measured in our laboratory. Furthermore, there was great variability of the prolactin response to alcohol among the subjects studied. It is conceivable, however, that this variability indicates that certain individuals could constitute a group in which a stimulatory effect of alcohol might be of clinical significance. Our data provide no information on the effect of long-term alcohol consumption, the effect of concurrent alcohol administration on thyroliberin-induced prolactin release or the prolactin response to acute alcohol administration in subgroups of alcoholics such as those studied by Van Thiel *et al.* [12].

The modest increases in plasma prolactin found in this study should be considered in relation to the prolactin response observed under other conditions. Among our subjects, psychic stress (anxiety) and physical stress (emesis) were apparently responsible for much greater increases in plasma prolactin than an intoxicating dose of alcohol per se. These stress related increases fall within the range of prolactin increments reported by Noel *et al.* [6] in a variety of stressful conditions. Other pharmacological agents, including dopamine receptor blocking drugs [3] and opiates [8] are considerably more potent than alcohol in stimulating prolactin secretion. Even strenous exercise [6] and sleep [9] appear to be associated with greater prolactin increments above basal levels.

The experiments of Thorner *et al.* [7] suggest a parsimonious explanation for the small increase of about 31% in plasma prolactin that correlated with peak blood alcohol levels of 95–110 mg/dl in our study. At 500 mg ethanol/dl, the lowest concentration employed in their perfused preparation of isolated rat pituitary cells, prolactin release was increased 32%. At the present time, however, neither our data nor those of others provide more than a speculative basis upon which to suggest mechanistic explanations.

Although the small positive prolactin response to acute alcohol administration reported here differs from earlier negative findings from other laboratories, our conclusion remains essentially the same—alcohol per se probably does not have any physiologically meaningful influence on plasma prolactin in normal men.

ACKNOWLEDGEMENT

This study was supported in part by NIDA Grant No. DA 01676.

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